“Refractory” Eosinophilic Airway Inflammation in Severe Asthma

Effect of Parenteral Corticosteroids

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It has been suggested that patients with refractory eosinophilic airway inflammation represent a separate “eosinophilic” asthma phenotype associated with increased morbidity and a poor prognosis. To investigate whether persistent eosinophilia in these patients is a fixed feature or can still be modified by treatment, we investigated the effect of high-dose intramuscular corticosteroids on eosinophils in induced sputum. Twenty-two patients with stable severe asthma (15 women, aged 21–73 years) participated in this double-blind, placebo-controlled study. All were using inhaled corticosteroids (> 1,600 µg/day) or chronic oral prednisone. They were included if the percentage of eosinophils in induced sputum was above the upper limit of normal (> 2%). Two weeks after treatment with triamcinolone, but not placebo, sputum eosinophils almost completely disappeared from a median of 12.6–0.2% (p < 0.001). In 82% of patients, no eosinophils could be observed at all. In addition, the rescue medication score decreased from 1.4 to 0.8 (p < 0.001). We conclude that persistent sputum eosinophilia despite extensive antiasthma treatment is not a refractory phenomenon but is still sensitive to high-dose systemic corticosteroids. This implies that these patients with severe asthma need additional or alternative antiinflammatory treatment to combat the eosinophilia and associated poor prognosis.

Keywords: asthma; eosinophilia; glucocorticoids; phenotype; sputum; severity of illness index

There is now accumulating evidence that patients with bronchial asthma are heterogeneous with respect to the type of inflammation in the airways and the response to antiinflammatory therapy (1–4). Most patients with asthma have eosinophilic airway inflammation with good response to treatment with inhaled corticosteroids (5). However, a relatively large proportion of adult asthma cases is characterized by neutrophilic airway inflammation (4, 6), in particular those with severe disease (7–9). These patients are more difficult to treat and often require high doses of inhaled or oral corticosteroids to control their disease. Remarkably, bronchial biopsy and bronchoalveolar lavage studies have shown that in a subgroup of patients with severe asthma such neutrophilic airway inflammation is accompanied by persistent eosinophilic inflammation (10, 11).

Eosinophilic inflammation in the airway mucosa that persists despite the use of high doses of inhaled corticosteroids or oral corticosteroids has been observed in several studies (9, 12–18) and has been implicated by Wenzel and colleagues as a feature of a separate asthma phenotype, associated with poor asthma prognosis (10, 12). Indeed, studies have shown that eosinophilic inflammation despite vigorous antiasthma treatment is associated with remodeling of the airways, impaired lung function, and near-fatal asthma attacks (10, 19, 20).

Why eosinophilia is persisting in these patients is unknown. Moreover, it has not been investigated whether persistent eosinophilia is a permanent characteristic of a specific phenotype of severe asthma or can be modulated by more intensive treatment.

In this study, we investigated the effect of high-dose intramuscular corticosteroids on sputum eosinophilia in a subgroup of patients with severe asthma featuring persistent eosinophilia despite extensive antiinflammatory treatment. In addition, the effect of intramuscular corticosteroids on asthma symptoms, lung function, peripheral blood eosinophils, and nitric oxide in exhaled breath was examined. The results of this study have been published previously in the form of an abstract (21).

METHODS

Patients

The patients in this study were part of a cohort of severe asthma patients participating in studies aimed at identifying risk factors of asthma severity (20). From the original cohort of 152 patients, 22 nonsmoking outpatients meeting the criteria of severe bronchial asthma (22) were selected. The patients were included if they were clinically stable for at least 4 weeks and while the percentage of eosinophils in their sputum was above the upper limit of normal (≥ 2%) (23). All patients used 1,600–6,400 µg/day beclomethasone equivalent and long-acting bronchodilators for more than 1 year and had had at least one (median, 4; range, 1–7) course of oral corticosteroids during the past year or 5 mg or more of oral prednisone daily. Patients with contraindications for systemic corticosteroids were excluded. The study was approved by the hospital medical ethics committee, and all patients gave written informed consent.

Design

The study had a randomized, parallel, double-blind, placebo-controlled design. At baseline (Visit 1), patients’ characteristics were documented, and Borg score, postbronchodilator FEV₁, and level of exhaled nitric oxide were assessed. A blood sample was taken, and sputum was induced. If the sputum eosinophil percentage exceeded 2%, patients were randomized to one of the two treatments, with stratification for daily use of oral steroids (strata yes/no), and level of sputum eosinophil percentage (strata 2–10% or > 10%). After 1 week (Visit 2), one single intramuscular injection of 3 ml (40 mg/ml) long-acting triamcinolone acetonide (Kenacort-A 40; Bristol-Myers Squibb, Woerden, The Netherlands) or matched placebo (3 ml NaCl10.9%) was given. Intramuscular administration was chosen instead of a course of oral corticosteroids to avoid any confounding by noncompliance with therapy. Two weeks thereafter (Visit 3), the measurements performed at Visit 1 were repeated. A diary was completed by the patients during the first and third weeks of the study.

Measurements

Postbronchodilator FEV₁ was assessed (24) 30 minutes after the inhalation of 400 µg salbutamol, and exhaled nitric oxide measurements were
performed as previously described (25). Sputum was induced according to a validated protocol (15), and whole sputum samples were processed (26). Peripheral blood eosinophil and neutrophilic percentages were measured by standard automated cell counter. A Borg scale (0–10) was used to rate the patient’s dyspnea (27) by asking this: “When thinking about daily activities regularly causing dyspnea, how severe was your dyspnea during the last week as compared with the severest dyspnea you ever experienced?” Additionally, on diary cards, the severity of daytime dyspnea, cough, sputum production and limitation in activities, and nocturnal dyspnea and cough were registered on a scale ranging 0 to 3. The cumulative symptom score per day (range 0–18) was analyzed. Finally, rescue medication score was expressed as the mean number of as-needed puffs of β2-agonist or ipratropium bromide per day.

Statistical Analysis
Eosinophil and neutrophil percentages in blood and sputum were log-transformed before statistical analysis. Two-tailed paired and unpaired t tests, Wilcoxon rank tests, or chi-square analyses were used for between-group analyses at baseline and for the analysis of within-group treatment effects.

Between-group changes were explored using analysis of variance with treatment as between-patient factor and age, age at onset of asthma, asthma duration, and baseline value of the measurement as covariate (28). All analyses were performed using SPSS for Windows, version 10.0 (SPSS, Inc., Chicago, IL).

RESULTS
Patient Characteristics
Eleven of the 22 participating patients were randomized to triamcinolone-treatment, whereas the remaining 11 patients received placebo. There were no significant differences between the groups in age and asthma duration (Table 1), with a trend toward a higher age at the onset of asthma in the placebo-treated patients (p = 0.07). Also sex, smoking history, medication use, and atopy were equally distributed between the two groups. Baseline treatment remained unchanged during the study. There were no dropouts.

Effects on Sputum Eosinophils
The intramuscular injection of triamcinolone/placebo was well tolerated in all patients, and no serious side effects were reported. At baseline, sputum eosinophil percentages in the triamcinolone-treated group were not different from those in the placebo-treated group (median [range] 12.6% [2–64%] vs. 17.0% [2–30%], respectively; p > 0.5). In addition, high-sputum neutrophil percentages were found in the triamcinolone group as well as in the placebo-treated patients (median [range] 70.8% [28–92%] vs. 58.0% [50–85%], p > 0.5).

Two weeks after the intramuscular injection, sputum eosinophilia in the triamcinolone-treated group was almost abolished to a median (range) percentage of 0.2 (0–5.8), whereas in the placebo group, the eosinophil percentage remained unchanged at 24.8 (5–44) (p = 0.01) (Figure 1). In all triamcinolone-treated patients, sputum eosinophilic decreased to normal levels (0–2%), except for one patient in whom the percentage decreased from 41.4 to 5.8. In contrast, the percentage neutrophils in induced sputum did not change significantly after triamcinolone treatment. At the final visit, three of the triamcinolone-treated patients and two patients of the placebo group were not able to cough up an adequate sputum sample anymore.

Effects on Other Parameters
There were no significant differences in baseline values between the two groups (p > 0.2), except for a lower diary score (p = 0.02) and a trend toward a lesser use of rescue medication (p = 0.09) in the triamcinolone group as compared with placebo (Table 2). Triamcinolone treatment resulted in an improved postbronchodilator FEV1, with a median (range) change of 11.8% (0–34%) from baseline versus a change of −5% (−44–22%) in the placebo-treated patients (p = 0.02) (Figure 2, left). Exhaled nitric oxide levels decreased in the triamcinolone-treated group from 21.5 ppb (2–55 ppb) to 7.9 ppb (2–32 ppb) (p = 0.02) but not in the placebo-treated group. The use of rescue medication decreased after triamcinolone but not placebo treatment (difference between groups, p < 0.01) (Figure 2, right), and the change in Borg score was higher in the triamcinolone group as compared with the placebo group (p = 0.01). However, the change in diary symptom score was not different between the groups (p = 0.16). Within the triamcinolone-treated group, changes in sputum eosinophils were correlated with changes in postbronchodilator FEV1 (r = 0.8, p = 0.03) and use of rescue medication (r = 0.8, p = 0.03), but not with changes in Borg scores (p = 0.73), diary symptom scores (p = 0.86), or exhaled nitric oxide levels (p = 0.53). In the peripheral blood samples, the eosinophil percentage decreased, and neutrophil percentage rose after an injection with triamcinolone, whereas placebo did not change the cell differentiation (Table 2). Similar results were obtained with or without correction for differences in age, asthma duration, and baseline values.

DISCUSSION
This study shows that persistent sputum eosinophilia despite extensive antiinflammatory treatment in patients with severe asthma is not a refractory phenomenon but is still sensitive to high-dose systemic corticosteroids. In fact, sputum eosinophilia was almost entirely abolished by the administration of high doses of parenteral corticosteroids, whereas sputum neutrophilia remained unchanged. The normalization of sputum eosinophilia

<table>
<thead>
<tr>
<th>TABLE 1. PATIENT CHARACTERISTICS</th>
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<tbody>
<tr>
<td><strong>Triamcinolone</strong></td>
</tr>
<tr>
<td>(n = 11)</td>
</tr>
<tr>
<td>Age, yr*</td>
</tr>
<tr>
<td>Sex, M/F</td>
</tr>
<tr>
<td>Age at onset asthma, yr†</td>
</tr>
<tr>
<td>Asthma duration, yr†</td>
</tr>
<tr>
<td>Pack-years†</td>
</tr>
<tr>
<td>Dose inhaled steroids, μg/day*</td>
</tr>
<tr>
<td>Maintenance oral steroids, n (%)</td>
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<td>Positive RAST, n (%)</td>
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* Mean ± SD.
† Median (range), p values for differences between groups.
was accompanied by an improvement in FEV<sub>1</sub> and a decreased use of rescue medication. These results suggest that patients with persistent eosinophilia are candidates for alternative therapeutic approaches to target the eosinophilic inflammation and to improve long-term outcome.

This study provides us with a number of novel findings that have both scientific and clinical implications. First, it suggests that the term “eosinophilic phenotype” of severe asthma should not be handled as a fixed disease entity but rather as an indication of disease activity and persistence. Apparently, airway eosinophilia in these patients is not static. Therefore, the classification of severe asthma phenotypes in “eosinophilic” or “non-eosinophilic” (10, 12) probably needs to be reconsidered. This has potential implications for future genetic studies because it has now become clear that persistent airway eosinophilia per se is probably not associated with a specific genotype in severe asthma (29).

Second, this study shows that the elevated percentages of eosinophils in sputum normalized completely after a high dose of parenteral corticosteroids, suggesting that these patients may be relatively insensitive to steroids but do not represent a distinct subgroup of patients with complete steroid resistance at a cellular level. Relative insensitivity to corticosteroids in patients with severe airway inflammation is conceivable and might result from allergen- or infection-induced cell activation or chronic exposure to medications such as β-agonists or corticosteroids (30). If this is the major cause of persistent eosinophilia, alternative antiinflammatory and immunomodulating approaches should be considered to control the inflammatory process further (31).

Third, the study shows that high-dose parenteral corticosteroids have no effect on airway neutrophilia in this category of patients. Before treatment we observed a prominent neutrophilic inflammation that did not change after steroid therapy. This suggests that neutrophilia in these patients is not induced by corticosteroid therapy but is inherent to the type of inflammation in severe asthma (13). Whether neutrophils contribute to steroid resistance or are associated with underlying causes of steroid resistance remains an important question (4).

Fourth, the study is clinically important. Patients with severe asthma are to be treated according to Step 4 of the international guidelines (32). This implies the use of oral corticosteroids. Most patients and doctors are reluctant to use these drugs continuously because of the serious side effects. This is why some patients prefer to remain symptomatic instead of using oral corticosteroids on a daily basis. This study shows that both the intensity of airway inflammation and the level of symptoms and lung function can (and should) be improved by intensifying treatment and/or administering it via the systemic route. Regular monitoring of sputum eosinophils to adjust antiinflammatory treatment might be necessary in these patients to prevent severe exacerbations and airway fibrosis (33, 34).

The question remains why eosinophilia persists in these patients with severe asthma despite high doses of inhaled corticosteroids. Apart from relative steroid resistance (30), an alternative explanation might be that parenteral corticosteroids were able to reach regions of the airways that are hardly accessible to inhaled corticosteroids, such as the peripheral airways (35) or upper airways including the paranasal sinus (36). These drugs also reach the bone marrow and may have influenced eosinophopoiesis being upregulated by hemopoietic factors produced by inflamed tissue (37). Also, relative undertreatment caused by impaired perception of dyspnea in this category of patients with severe asthma might have been the case (38). Finally, noncompliance with treatment might be a possible explanation of persistent eosinophilic inflammation in some patients, which is suggested by insufficiently suppressed serum cortisol levels in one patient on oral corticosteroids (data not shown). However, this is not the explanation in the majority of patients.

In conclusion, this study shows that in patients with severe asthma with sputum eosinophilia despite extensive antiasthma treatment, a high dose of intramuscular corticosteroids results in normalization of sputum eosinophilia, associated with a decreased use of rescue medication and an improvement of postbronchodilator FEV<sub>1</sub>. This implies that persistent eosinophilia despite treatment is a phenomenon that can be overcome by intensifying the antiinflammatory treatment. If persistent eosino-

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**TABLE 2. DIFFERENCES BETWEEN TRIAMCINOLONE AND PLACEBO TREATMENT**

<table>
<thead>
<tr>
<th></th>
<th>Triamcinolone (n = 11)</th>
<th>Placebo (n = 11)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>2 Weeks</td>
</tr>
<tr>
<td>Borg score</td>
<td>2.0 (0–5)</td>
<td>0.5 (0–3)</td>
</tr>
<tr>
<td>Diary score</td>
<td>3.5 (1–6)</td>
<td>1.4 (0–7)</td>
</tr>
<tr>
<td>Rescue med score</td>
<td>1.4 (0–8)</td>
<td>0.8 (0–3)</td>
</tr>
<tr>
<td>Pb FEV&lt;sub&gt;1&lt;/sub&gt;, %pred</td>
<td>73.8 (42–107)</td>
<td>88.3 (44–119)</td>
</tr>
<tr>
<td>NO, ppb</td>
<td>21.5 (2–55)</td>
<td>7.9 (2–32)</td>
</tr>
<tr>
<td>Blood eo, %</td>
<td>6.2 (1–18)</td>
<td>1.3 (0–5)</td>
</tr>
<tr>
<td>Sputum neutro, %</td>
<td>12.6 (2–64)</td>
<td>0.2 (0–6)</td>
</tr>
<tr>
<td>Blood neutro, %</td>
<td>54.1 (39–77)</td>
<td>71.7 (44–80)</td>
</tr>
<tr>
<td>Sputum neutro, %</td>
<td>70.8 (28–92)</td>
<td>75.1 (35–94)</td>
</tr>
</tbody>
</table>

*Definition of abbreviations: eo = eosinophils; med = medication; neutro = neutrophils; NO = nitric oxide; Pb = postbronchodilator.

Median (range) values; within-group p values for changes within group, between-group p values for differences in changes between groups, using analysis of variance, with age, age-at-onset asthma, asthma duration, and baseline value as covariates.
phlic airway inflammation is causally related to more severe asthma, remodeling of the airways, and a poor prognosis of the disease, then alternative antiinflammatory therapies are urgently needed. Ideally, this would imply the use of novel potent immunomodulatory agents that are safe and capable of reaching the peripheral airways, the paranasal sinus, as well as the bone marrow.

Conflict of Interest Statement: A.T.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; P.J.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.F.R. has consulted, participated in Advisory Board Meetings, and received lecture fees from AstraZeneca, Boehringer, Pfizer, Novartis, AltanaPharma, MSD, and GlaxoSmithKline; E.H.B. participated in an Advisory Board Meeting of Merck, Sharpe, and Dohme. The Department of Pulmonary Diseases of Leiden University has received grants from AltanaPharma ($222,616), Novartis ($90,640), Bayer Sharpe, and Dohme. The Department of Pulmonary Diseases of Leiden University has received grants from AltanaPharma ($222,616), Novartis ($90,640), Bayer Sharpe, and Dohme. The Department of Pulmonary Diseases of Leiden University has received grants from AltanaPharma ($222,616), Novartis ($90,640), Bayer Sharpe, and Dohme. The Department of Pulmonary Diseases of Leiden University has received grants from AltanaPharma ($222,616), Novartis ($90,640), Bayer Sharpe, and Dohme. The Department of Pulmonary Diseases of Leiden University has received grants from AltanaPharma ($222,616), Novartis ($90,640), Bayer Sharpe, and Dohme. The Department of Pulmonary Diseases of Leiden University has received grants from AltanaPharma ($222,616), Novartis ($90,640), Bayer Sharpe, and Dohme. The Department of Pulmonary Diseases of Leiden University has received grants from AltanaPharma ($222,616), Novartis ($90,640), Bayer Sharpe, and Dohme.

Acknowledgment: The authors thank M.C. Timmers and H. van der Veen for technical assistance and the chest physicians of the participating hospitals for their cooperation (P.I. van Spiegel, G.Visschers, Slotervaart Hospital, Amsterdam; H. van der Heijden, Rode Kruis Hospital, Beverwijk; B. J. M. Pannekoek, Reinier de Graaf Gasthuis, Delft; H. H. Berendsen, K. W. van Kralingen, Bronovo Hospital, Den Haag; H. G. M. Heijerman, A. C. Roldaan, Leyenburg Hospital, Den Haag; A. H. M. van der Heijden, Spaarne Hospital, Heemstede; H. C. J. van Klink, Diaconessenhuis, Leiden; C. R. Apaag, St. Antoniushove, Leidschendam; and A. Rudolphus, K. Y. Tan, St. Franciscus Gasthuis, Rotterdam).

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